CB and ex vivo CB as well as peripheral blood mononuclear cells (PBMC) as controls. T cell receptor spectratyping was performed before and after expansion with aim to determine usage of different TCR Vbetas.

Results: T cells expanded with a median of 148-fold over a period of 8–11 days. Expanded preparations contained 99% of CD3+ cells without signs for significant expansion of CD19+ or CD3-/CD56+ cells. CD4/CD8 ratio of expanded T cells was not altered significantly when compared to CB ex vivo (a mean of 1,7 to 2,02, respectively). The majority of T cells were positive for αβ T cell receptor (93%). Up to 85% of expanded T cells were CD25+, 3% of CD3+ fraction were CD25+/FoxP3+. Expanded T cells, when stimulated with CD3-beads, produced IL-2, IFN-g, TNF-a in a cell to bead ratio in a dose-dependant manner at higher levels, comparaed to peripheral T cells and CB ex vivo T cells. TCR spectratyping has shown a polyclonal pattern of TCR Vbeta-gene usage in the expanded T cell pool.

Conclusions: We have successfully set up a clinically feasible system of T cell expansion for use as DLI after CBT. The expansion procedure has not introduced major phenotypic changes when compared to CB cells ex vivo. Expanded cells are functional in terms of cytokine production and display no oligoclonal pattern of TCR usage. Expanded CB T lymphocytes may serve as a possibility for DLI after CBT.

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GRAFT-VERSUS-HOST REACTIONS TARGET HEMATOLYMPHOID ORGANS LEADING TO ALTERATIONS IN HEMATOPOIETIC RECONSTITUTION AND DYSFUNCTIONAL IMMUNITY

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The pathophysiology of chronic (c) Graft-versus-Host Disease (GVHD) is poorly understood. The protean nature of the syndrome suggests more complex mechanisms than pure T cell (TC) effects. We analyzed cellular infiltrates of hematolymphoid tissues (bone marrow (BM), spleen, lymph nodes (LN), thymus, liver) after hematopoietic cell transplantation (HCT) in 3 minor-mismatched mouse models of GVHD. Myeloablated BALB.B (H2b), BALB/c (H2d), and BALB.K (H2k) mice were given purified hematopoietic stem cells + splenocytes (SP) from C57BL/6 (B6), B10.D2, or AKR/J donors, respectively. BALB.B recipients developed acute and chronic GVHD. BALB/c mice showed skin lesions on ears and tail, which resolved by d50, but no systemic or late symptoms. Despite a lower SP dose BALB.K hosts developed fulminant GVHD. Differences in type and kinetics of cellular infiltration of organs were observed between the strains. In all 3 models BM was a major site of donor (do) TC infiltration early post-HCT. While B cell (BC) reconsititution was severely delayed BALB.B BM contained ~50% doTC, which were in ~60% CD8+ (effector memory (EM) phenotype), and H60 tetramer-reactive in up to 24%. At a low level doTC persisted long-term in the BM. BALB/c recipients of B10.D2 grafts had better BC regeneration and less infiltrating doTC (40%). The latter were CD4/8 balanced and normalized promptly (d50 <3%). In contrast, BALB.K mice given AKR/J grafts, had prominent CD4 (EM) doTC infiltrates. The liver was a main target of GVH in BALB.B recipients: 14d post-HCT liver MNC contained 60–80% doTC, 30% reactive to H60. Low levels of infiltration were observed long-term. In BALB/c livers Mac1/Gr1+ cells predominated (<60%), while doTC infiltration was less severe and resolved completely. BALB.K livers contained ~80% doTC, mostly CD4+. Thymuses, spleen and LN of BALB.B and K recipients were hypoplastic, hypocellular and infiltrated by doTC with a decreased CD4/8++ fraction. BALB.K LN had predominantly CD4 doTC and lacked BC, while BALB.B LN contained CD8>CD4. Thymuses, spleen and LN of BALB/c recipients normalized after some initial doTC infiltration. In conclusion, hematolymphoid organs can be GVH targets, which is associated with impaired hematopoietic and immune reconstitution. Damage of these tissues may disturb normal immunity, and via dysfunctional immune education indirectly contribute to autoimmune-like phenomena as seen in chronic GVHD.

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FEASABILITY OF EXTRACORPOREAL PHOTOPHERESIS IN MANAGING PATIENTS WITH BRONCHIOLITIS OBLITERANS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Introduction: Bronchiolitis obliterans (BO) is a serious, often fatal complication of allogeneic stem cell transplantation (SCT). Most patients do not respond to conventional immunosuppressive therapy. We retrospectively reviewed records of patients with BO treated with extracorporeal photopheresis (ECP), given its efficacy in managing chronic corticosteroid refractory cutaneous, oral, and hepatic graft versus host disease (GVHD).

Patients and Methods: Forty two patients were treated at our center for chronic GVHD with ECP from 2003–2007. Thirteen patients (31%) met diagnostic criteria for BO defined as the presence of pulmonary symptoms, hypoxemia and one of the following 1) decrease in FEV1 by >20% or 2) air-trapping, small airway thickening or bronchiectasis on lung CT without evidence of an infection. ECP was performed on 2 consecutive days every 2 weeks for the first 4 months and less frequently thereafter.

Results: Nine of thirteen patients met criteria due to decrease in FEV1 and lung changes on CT; 4 due to radiographic findings alone. ECP was initiated at a median of 954 days (range, 173–2122 days) after allogeneic SCT (11 matched related donors, 2 unrelated donors). All but one patient had failed prior corticosteroid-based immunosuppressive therapy. With a median follow-up of 782 days from diagnosis of BO, 11/13 (85%) of the patients are alive. Seven of thirteen (54%) patients had a 50% decrease in steroid dosage without deterioration of pulmonary function; 3/13 (23%) patients had improvement of radiographic abnormalities; 4/7 (57%) patients with oxygen requirements prior to initiation of ECP were completely titrated off oxygen. No patients had a decline in pulmonary function tests despite steroid withdrawal. Only 3 patients experienced grade III or higher adverse events related to the indwelling catheter (3 infectious, 1 bleeding and 2 thromboses). Two of thirteen patients died, one due to progression of BO and 1 from pneumonia. Our observed survival rate of 85% in patients with BO is in contrast to the 44–73% survival rate noted in prior studies using treatment with conventional immunosuppressive therapy.

Conclusion: In this retrospective study, we demonstrate the feasibility of using ECP to manage BO occurring after allogeneic SCT. ECP should be considered as early second-line therapy in patients developing BO following SCT; however, further study is warranted to confirm these results.

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BONE MARROW AND INTESTINAL BUT NOT PERIPHERAL BLOOD EOSINOPHILIA PREDICTS ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) AFTER HEMATOPOETIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING (RIC-HCT)

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Objectives: Acute GvHD remains a severe complication after RIC-HCT. We report here the predictive value of bone marrow and intestinal eosinophilia in patients transplanted from 1999 to 2005 by using RIC HCT.

Patients and methods: One hundred and fifteen patients (55 female) with haematological malignancies with the median age of 59 years (range 21 to 75) were treated with RIC-HCT for AML (n = 76), ALL (n = 7), CML (n = 13) and MDS (n = 19). Conditioning regimen consisted of fludarabine at day -4 to -2 and 2 Gy total body irradiation at day 0 followed by the infusion of HCT of an related (n = 30), allele matched unrelated (n = 76) or mismatched (n = 9) donor and treatment with MMF and CSA. Bone marrow and peripheral blood evaluation was performed before and on day +28 after RIC-SCT; Eosinophilia was graduated from 0 to 4. Systematic colon and/or duodenal biopsies have been performed immediately after first symptoms of gut GvHD. Histological criteria of gut GvHD were assessed and tissue eosinophils analysed within the lamina propria and lamina submucosa. The density of

eosinophils in bone marrow, gut and peripheral blood were analysed by using SPSS.

Results: The incidence of acute GvHD was 51% (36% grade 1, 27% grade 2, 15% grade 3 and 22% grade 4 out of it). One organ aGVHD was diagnosed in 42 (skin n = 36, gut n = 4, liver n = 2), two-organ aGvHD in 10 and tree-organ aGvHD in 7 patients. The density of bone marrow eosinophils was increased after RIC-SCT in comparison to before it (6% vs 3.4%, $p<0.001$, respectively). Bone marrow eosinophilia after RIC-SCT was found in 66% of patients with aGvHD and was a significant predictive factor ($p<0.03$) for developing aGvHD. However, eosinophil density did not correlated with aGvHD severity. Peripheral blood eosinophilia was not predictive for developing aGvHD. Intestinal eosinophils were found in 15 of 20 patients with clinical signs of gut GvHD, which has been consequently histologically proven in 13 patients (87%, $p<0.02$). The degranulation of eosinophils, determined by using anti-MBP was present in all patients with gut aGvHD.

Conclusions: Both gastrointestinal tract and bone marrow tissue eosinophilia after RIC-SCT predict aGvHD. In addition, gut eosinophil density and degranulation were increased in patients with higher grades of aGvHD thus indicating their role as a biological marker of GvHD. To our knowledge this is the first study showing that tissue eosinophil density might be a predictive marker for aGvHD after RIC-SCT.

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IMMUNOMODULATORY EFFECTS OF VITAMIN D: IMPLICATIONS FOR THE TREATMENT OF GRAFT VERSUS HOST DISEASE

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Allogeneic transplantation is uniquely curative for some patients with hematologic malignancies. However, morbidity and mortality due to graft versus host disease remain substantial. Persistence of host dendritic cells (DCs) in the early post transplant period plays a role in the activation of alloreactive lymphocytes and the risk of GVHD. A focus of research involves the manipulation of DC recovery post-transplant to minimize activation of alloreactive lymphocytes while preserving the graft versus disease effect. Vitamin D is a hormone involved in bone metabolism. More recently, vitamin D has been shown to have immunomodulatory effects. We evaluated the effect of vitamin D on the phenotypic and functional characteristics of DC and T cell populations. Peripheral blood mononuclear cells were isolated from leukopaks obtained from normal donors. DCs were generated by culturing the monocyte enriched adherent fraction with GM-CSF and IL-4 for 5 days, followed by TNFa for 48 hours. DCs were generated in the presence and absence of 10nM of 1,25 hydroxyvitamin D. Mean expression of the costimulatory molecule CD80 and the maturation marker CD83 decreased from 60% to 37% and 53% to 27% respectively in the presence of vitamin D (N = 3). To assess the effect of vitamin D on the functional potency of DCs as antigen presenting cells, the capacity of DCs to stimulate allogeneic T cell proliferation in the presence of vitamin D was determined. Mature DCs were cultured with allogeneic T cells at a ratio of 1:10. After 5 days, cocultures were pulsed with tritiated thymidine overnight. The addition of vitamin D resulted in a blunted T cell proliferative response, with mean SI that decreased from 13 to 5 (n = 10). Similarly, the addition of vitamin D to a coculture of DCs and autologous T cells resulted in a 50% reduction in the T cell proliferative response to tetanus toxoid, a recall antigen. In addition, T cells stimulated by allogeneic DCs in the presence of vitamin D were polarized to secrete Th2 cytokines. The presence of vitamin D did not induce FOXP3 expressing regulatory T cell populations. These data suggest that exposure to vitamin D exerts a tolerizing influence on T cells mediated by its impact on antigen presenting cells. Vitamin D may therefore have a role in the prevention and treatment of graft versus host disease. A clinical trial evaluating the use of vitamin D in the early post-transplant period for the prevention of GVHD is planned.

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ALLOGENEIC VERSUS AUTOLOGOUS STEM CELL TRANSPLANTATION (SCT) FOR FOLLICULAR LYMPHOMA (FL). THE JAMES COMPREHENSIVE CANCER CENTER EXPERIENCE

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Majority of the reported studies comparing allogeneic versus autologous SCT in patients with FL have short follow-up (median = or< 5-yrs). Patients with histologically confirmed FL (n = 117) undergoing SCT between 1985 and 2007 were eligible for this study. Patient characteristics including age, stage, LDH, number of prior therapies, remission status at SCT [CR, PR, untreated relapse (REL), refractory (REF)], and transplant characteristics were recorded. The median age was 49 years (range 23–71). 36 patients underwent allogeneic SCT (including 10 patients receiving reduced intensity conditioning), while 81 patients underwent autologous SCT. The median number of prior treatments for autologous and allogeneic SCT groups were 2 (range 1–5) and 3 (range 1–7) respectively. Median follow-up is 7-years. 5-year OS following autologous SCT for patients in CR1/PR1, CR>1/PR>1 and relapsed or refractory disease was 79%, 71% and 53% respectively. The respective OS for allogeneic SCT was 51%, 75%, and 49%. Relapse rates were lower following allogeneic SCT versus autologous SCT at 27% versus 55% respectively. 5 year progression free survival (PFS) was higher following allogeneic SCT at 46% versus 38%. Higher non-relapse mortality (NRM) with allogeneic SCT (25% versus 11%) resulted in a 5 year OS favoring autologous SCT (67% versus 57%). With prolonged follow-up a plateau was seen in allogeneic SCT curve at around 3-yrs, while autologous SCT patients continued to experience events. Hence the estimates of 10-yr OS for allogeneic SCT patients was 57% (unchanged from 5-yr OS), compared to 48% for autologous SCT. Our study shows that although the early results of allogeneic SCT (mostly with myeloablative conditioning) are negatively impacted with associated high NRM, it produces durable remissions, with eventual appearance of trends of improved survival with prolonged follow-up.

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PHARMACOKINETICS (PK) OF IV AND PO MYCOPHENOLATE MOFETIL (MMF) IN AGE ADJUSTED PEDIATRIC AND ADOLESCENT ALLOGENEIC STEM CELL TRANSPLANT (ALLOSCT) RECIPIENTS: SIGNIFICANTLY HIGHER CL_{SS} AND V_{SS} IN PATIENTS <6 YEARS OF AGE

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Children undergoing AlloSCT exhibit higher MMF dose requirements and significant interpatient variability in mycophenolic acid (MPA) PK (Jacobson P et al, *Ped* 2008). The objective of this study is to evaluate effects of age and conditioning intensity on the PK of MMF in pediatric AlloSCT recipients. From 1/04-5/08 we enrolled 38 pts: med age 8 yrs (0.33–16); M:F = 20:18; 22/16 malignant/non; 17/21 myeloablative (MA)/non-ablative (NMA); 16/22 related/unrelated. Cohort 1 (<6 yrs) n = 14; 2 (6–12 yrs) n = 10; 3 (12–16 yrs) n = 12. GVHD prophylaxis: tacrolimus Day –1 (5–20 ng/mL) and MMF 900 mg/m^2 IV Q6H starting on Day +1, then converted to PO (same dose) after Day +14. MPA serum samples were drawn on Days +1, +7, +14 (IV phase) and twice between Day +45-+100 (PO phase) at hour 0, 0.5, 1, 2, 3, 4, 6 post-dose. MPA plasma concentrations were determined by reverse-phase HPLC and LC/MS/MS. Non-compartmental PK analysis of total MPA was performed. Median time to myeloid and platelet engraftment was 18 and 31 d, respectively. KM probability of Grade II-IV acute GVHD (aGVHD) and extensive chronic GVHD